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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OCT - 5 '93

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: **DICAMBA: Developmental Toxicity Study in Rabbits**

FROM: Steven L. Malish, Ph.D., Toxicologist S.L. Malish 10/1/93
Section IV, Toxicology Branch II
Health Effects Division (H7509C)

TO: Robert Taylor/Vickie Walters
Product Manager (25)
Registration Division

THRU: Jess Rowland, M.S., Acting Section Head Jess Rowland 10/1/93
Section IV, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D., Chief Marcia van Gemert 10/4/93
Toxicology Branch II
Health Effects Division (H7509C)

STUDY IDENTIFICATIONS:	**Submissions:**	**DP Barcodes:**	**Action Codes:**
	S436801	D189040	231
	S436802	D189042	251
	S436804	D189044	330

P.C. Code: 029801 **CASWELL No.:** 295

Registrant: Sandoz Crop Protection, Des Plains, Il.

ACTION REQUESTED: Review of Developmental Toxicity study in Rabbits with Dicamba [**MRID No: 424294-01**].

RESPONSE: A Data Evaluation Report [DER] for the above referenced study is attached. A summary is provided below.

cc: W. Waldrop/J. Coombs, Product Manager 71, Reregistration Division

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DEVELOPMENTAL TOXICITY STUDY 010617

1. DEVELOPMENTAL RANGE-FINDING STUDY

Impregnated New Zealand White rabbits were orally administered (by capsule) Dicamba, technical [90.5% a.i.] at doses of 0, 62.5, 125, 250, or 500 mg/kg/day during days 6 through 18 of gestation. Dams were sacrificed on gestation day 29.

Dicamba technical did not induce maternal or developmental toxicity at 62.5 mg/kg/day. Treatment related maternal toxicity was manifested by mortality, increased resorptions and reduction in the litter size at 500 mg/kg/day. Clinical signs occurred at 125, 250 and 500 mg/kg/day. The 500 mg/kg/day animal showed a decrease in body weight gain during days 6 to 19 of dosing; during the postdosing period body weight was only equivalent to that of the control but increased in the other dosage groups. The mean absolute [gm/day] and relative [gm/kg/day] maternal feed consumption decreased at 250 and 500 mg/kg/day during the dosing period. In the postdosage period, increased absolute and relative feed consumption occurred.

Cesarean sections revealed no treatment-related differences between treated and control groups, and no external malformations or variations were seen in any of the fetuses of the treated does. Based on the results of this study the NOELs and LOELs are:

MATERNAL TOXICITY NOEL = 62.5 mg/kg/day; LOEL = 125 mg/kg/day
DEVELOPMENTAL TOXICITY NOEL = 500 mg/kg/day; LOEL = not determined

Dose levels selected for the main study: 0 30, 150 or 300 mg/kg/day.

CORE CLASSIFICATION: Not applicable; range-finding study.

2. DEVELOPMENTAL TOXICITY STUDY

Inseminated New Zealand White rabbits were administered Dicamba technical [90.5% a.i.] by oral capsule at doses of 0, 30, 150 or 300 mg/kg/day during days 6 through 18 of gestation. No maternal toxicity was observed at 30 mg/kg/day. At 150 mg/kg/day, maternal toxicity was characterized by abortion and clinical signs. At 300 mg/kg/day, maternal toxicity was manifested by abortions, clinical signs, decreased body weight and body weight gain and decreased food consumption. Developmental toxicity at 300 mg/kg/day was manifested by irregular ossification of the nasal bones (internasals) of the skull; no developmental toxicity was seen at 30 or 150 mg/kg/day.

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Based on the results of this study the following NOELs and LOELs are established.

MATERNAL TOXICITY	NOEL =	30	mg/kg/day
	LOEL =	150	mg/kg/day
DEVELOPMENTAL TOXICITY	NOEL =	150	mg/kg/day
	LOEL =	300	mg/kg/day

CORE CLASSIFICATION: Minimum: This study satisfies the data requirements 83-3(b) for a Developmental Toxicity Study in rabbits and is acceptable for regulatory purposes.

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PRIMARY REVIEWER: Steven L. Malish, Ph.D. S.L. Malish 10/1/93
Section II, Toxicology Branch IV

SECONDARY REVIEWER: Jess Rowland M.S., Acting Section Head
Section II, Toxicology Branch IV Jess Rowland 10/1/93

DATA EVALUATION REPORT

DEVELOPMENTAL RANGE-FINDING STUDY

STUDY TYPE: Developmental Range-Finding GUIDELINE: N/A

IDENTIFICATIONS: MRID NO.: 424294-01 Caswell No.: 295

TEST MATERIAL: Dicamba technical

REGISTRANT: Sandoz Crop Protection, Des Plains, Il.

TESTING LABORATORY: Argus Research Laboratories, Inc.

STUDY IDENTIFICATION: 1819-004P

TITLE OF REPORT: Dosage-Range Development Toxicity Study (Embryo-Fetal Toxicity and Teratogenic Potential) Study of Technical Dicamba Administered orally via Capsule to New Zealand White Rabbits

AUTHOR: Alan M. Hoberman, Ph.D. **REPORT DATE:** September 24, 1991

SUMMARY: Impregnated New Zealand White rabbits were orally administered (by capsule) Dicamba, technical [90.5% a.i.] at doses of 0, 62.5, 125, 250, or 500 mg/kg/day during days 6 through 18 of gestation. Dams were sacrificed on gestation day 29.

Dicamba technical did not induce maternal or developmental toxicity at 62.5 mg/kg/day. Treatment related maternal toxicity was manifested by mortality, increased resorptions and reduction in the litter size at 500 mg/kg/day. Clinical signs occurred at 125, 250 and 500 mg/kg/day. The 500 mg/kg/day animal showed a decrease in body weight gain during days 6 to 19 of dosing; during the postdosing period body weight was only equivalent to that of the control but increased in the other dosage groups. The mean absolute [gm/day] and relative [gm/kg/day] maternal feed consumption decreased at 250 and 500 mg/kg/day during the dosing period. In the postdosage period, increased absolute and relative feed consumption occurred.

Cesarean sections revealed no treatment-related differences between treated and control groups, and no external malformations or

variations were seen in any of the fetuses of the treated does. Based on the results of this study the NOELs and LOELs are:

MATERNAL TOXICITY NOEL = 62.5 mg/kg/day; LOEL = 125 mg/kg/day
DEVELOPMENTAL TOXICITY NOEL = 500 mg/kg/day; LOEL = not determined

Dose levels selected for the main study: 0, 30, 150 or 300 mg/kg/day.

CORE CLASSIFICATION: Not applicable; range-finding study.

1. OBJECTIVE

The objective of this range-finding study was to establish appropriate dose levels of Dicamba, technical for the main study.

2. PROTOCOL

Groups of five impregnated female New Zealand White rabbits [7 months old] were given oral administrations of Dicamba technical (90.5% a.i., lot 5265110) at doses of 0, 62.5, 125, 250 and 500 mg/kg/day in clear gelatin capsules [Size 1] daily during days 6 through 18 of gestation. The control groups was given the same number of capsules as the 500 mg/kg/day group. Capsules containing the test substance were prepared daily based on the individual body weight of the animals.

Animals were observed for viability, clinical observations, abortions and premature deliveries. Dams were weighed on day 0, days 6 thru 18 [dosing] and days 24 and 29 of gestation. Individual food consumption was measured daily during gestation. Dams in each group were sacrificed on day 29 and postmortem examination included macroscopic examination of internal organs with emphasis on the uterus, uterine contents, position of each fetus in the uterus, and corpora lutea counts. The fetuses were sexed, weighed, examined, and discarded.

3. RESULTS

(i) Maternal Toxicity

- Treatment related maternal toxicity was manifested by mortality of 3 does at 500 mg/kg/day; one doe each at 250 and 500 mg/kg/day died from a dosing accident. No abortions occurred at any dose level.

- The pregnancy rate was 80% in the control, 100% at 62.5 mg/kg/day group and 80% in the 125, 250 and 500 mg/kg/day groups. The 1 doe that died at 500 mg/kg/day was not pregnant.

- Clinical signs of toxicity, manifested by hyper-reactivity [125, 250 and 500 mg/kg/day], myotonia, ataxia, excess

salivation, rales [250 and 500 mg/kg/day], lost righting reflex, decreased motor activity and red perioral substance [500 mg/kg/day].

- At 62.5 mg/kg/day, body weight or body weight gains were not affected versus the control. The 1 surviving 500 mg/kg/day animal showed a decrease in body weight gain during days 6 to 19 of dosing. During the postdosing period [days 19 to 29 of gestation], body weight increased versus the controls in animals at 62.5, 125 and 250 mg/kg/day; compensatory effects followed the post-dosing period. At 500 mg/kg/day the body weight gain in the 1 surviving animal was comparable to the control.

- The mean absolute [gm/day] and relative [gm/kg/day] maternal feed consumption showed a dose related decrease at 250 and 500 mg/kg/day during the dosing period. In the postdosage period, increased absolute and relative feed consumption values occurred.

- Necropsy observations occurred only in the does that died. Test compound related effects in each of the does that died presented pale and/or white stomachs; the 1 animal that died from a dosing accident at 500 mg/kg/day, revealed brown ulceration in the pyloric region of the stomach. Other necropsy observations were considered unrelated to the administration of the test substance and included trachea partially blocked by a capsule and/or test substance, hemorrhagic lungs and a parovarian cyst.

- No treatment-related effects were seen in the reproductive performances of the does except for the 1 surviving doe at 500 mg/kg/day which showed an increase in the mean number of resorption and a reduction in the live litter size.

(ii) <u>Developmental Toxicity</u>

- No developmental toxicity was observed at any dose level; treatment had no effect on corpora lutea, implantation sites, viable fetuses and early and late resorptions.

- Mean fetal body weights at all dose levels was similar to the control.

- No external malformations or variations were observed in any of the fetuses of treated females.

4. **CONCLUSION**

MATERNAL TOXICITY NOEL = 62.5 mg/kg/day; LOEL = 125 mg/kg/day; based on clinical signs, food consumption decreases and decreases in body weight.

DEVELOPMENTAL TOXICITY NOEL = 500 mg/kg/day; LOEL = not determined

Based on this study, dose levels selected for the main study were 0, 30, 150 or 300 mg/kg/day.

5. **CORE CLASSIFICATION** Not applicable; study not required.

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PRIMARY REVIEWER: Steven Malish, Ph.D, Toxicologist S.J. Malish 10/1/92
Section IV, Toxicology Branch II

SECONDARY REVIEWER: Jess Rowland, M.S., Acting Section Head Jess Rowland 10/1/92
Section IV, Toxicology Branch II

DATA EVALUATION REPORT

DEVELOPMENTAL TOXICITY STUDY

STUDY TYPE: Developmental Toxicity/Rabbits **GUIDELINE:** 83-3(b)

IDENTIFICATIONS: **Submission:** S436804 **DP Barcode:** D189044

MRID NO.: 424294-01 **Caswell No.:** 295

P.C. Code: 029801

TEST MATERIAL: Dicamba technical

REGISTRANT: Sandoz Crop Protection Corporation, Des Plains, Il

TESTING LABORATORY: Argus Research Laboratories, Inc.

STUDY IDENTIFICATION: 1819-004

TITLE OF REPORT: Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenicity Potential, Study of Technical Dicamba Administered Orally Via Capsule to New Zealand White Rabbits.

AUTHORS: Alan H. Hoberman, Ph.D. **REPORT DATE:** January 27, 1992

SUMMARY: Inseminated New Zealand White rabbits were administered Dicamba technical [90.5% a.i.] by oral capsule at doses of 0, 30, 150 or 300 mg/kg/day during days 6 through 18 of gestation. No maternal toxicity was observed at 30 mg/kg/day. At 150 mg/kg/day, maternal toxicity was characterized by abortion and clinical signs. At 300 mg/kg/day, maternal toxicity was manifested by abortions, clinical signs, decreased body weight and body weight gain and decreased food consumption. Developmental toxicity at 300 mg/kg/day was manifested by irregular ossification of the nasal bones (internasals) of the skull; no developmental toxicity was seen at 30 or 150 mg/kg/day.

Based on the results of this study the following NOELs and LOELs are established.

MATERNAL TOXICITY	**NOEL =**	**30 mg/kg/day**
	LOEL =	**150 mg/kg/day**
DEVELOPMENTAL TOXICITY	**NOEL =**	**150 mg/kg/day**
	LOEL =	**300 mg/kg/day**

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CORE CLASSIFICATION: Minimum: This study satisfies the data requirements 83-3(b) for a Developmental Toxicity Study in rabbits and is acceptable for regulatory purposes.

I. OBJECTIVE

The objective of this study was to assess the effects of the Dicamba on the embryonic and fetal development following oral administration to rabbits during the period of organogenesis.

II. MATERIALS AND METHODS

a. Test Material

Identity: Dicamba, technical
Lot No.: 52625110
Purity: 90.5% a.i.
Description: Off-white to light tan granular solid
Storage: cool, well ventilated area

b. Control

Identity: Opaque white gelatin capsule (Size 1))

c. Test Animals

Species/Sex: Female rabbits
Strain: New Zealand White [Hra:(NZW)SPF]
Body Weight on Gestation Day 0: 3.05 to 4.14 kg
Identification: Ear tags.
Acclimation: ≈3 weeks
Housing: Individually in stainless steel cages
Food: Purina Certified Rabbit Chow #5322, 180 gm/animal
Water: Tap water ad libitum
Environment: Temperature - 64 - 72°F; Humidity - 40-60%;
Light/Dark - 12 hr. cycle
Group Assignment: 19 or 20 inseminated females/group were randomly assigned to 1 control and 3 treatment groups.

d. Mating

The females were artificially inseminated with semen from proven male breeders of the same strain, obtained from the same source. The semen was diluted with normal saline and approximately 0.5 ml of the diluted semen was introduced into each female's vagina. Semen from one male was used to inseminate an equal number of females in each study group. Immediately following insemination, the females were administered HCG via the marginal ear vein. The day of insemination was considered Day 0 of gestation.

e. Preparation of Dose

Size 1 opaque capsules containing the test substance were prepared based on the individual body weight recorded on days 6, 9, 12 and 15. The control group was administered the same number of gelatin capsules as the high dosage.

f. Analyses of the Test Substance for Active Ingredient

The bulk test substance was analyzed for concentration of the active ingredient [2 separate experiments consisting of 5 separate assays performed on the same day]. The concentration (w/w) of the active ingredient was 90.5%. No significant differences were seen between the different experiments or assays.

g. Administration of Test Article

The test article was administered via oral capsule at doses of 0 (Control), 30, 150 or 300 mg/kg/day from day 6 through 18 of gestation. The control group received empty gelatin capsules.

h. Observations

All animals were observed twice daily for clinical signs of toxicity including physical or behavioral abnormalities. These rabbits were also observed for general appearance several times during the acclimatization period and on day 0. In addition, during the treatment period, the rabbits were observed immediately before each daily dose (days 6 to 18 of gestation) and within 60 minutes following dosing for detection of overt signs of toxicity. Additional observations were made once daily during the post-dosing period from days 19 to 29 of the presumed gestation.

Individual body weights were recorded weekly before insemination and on day 0 and then daily on days 6 through 29 of gestation.

Individual food consumptions were measured daily during the acclimatization and the study.

i. Termination

Females found dead or showing signs of abortion during the study were necropsied. All surviving does were sacrificed on gestation day 29.

j. Cesarean Section

The thoracic, abdominal and pelvic cavities were examined for gross lesions, and in the event of gross lesions, the tissues were preserved in neutral buffered 10% formalin. The uterus was removed from the body, examined externally, weighed and then opened for internal examination. Uteri that appeared to be from nonpregnant rabbits were stained with 10% ammonium sulfide to determine pregnancy status. Corpora lutea were counted, the number and placement of implantation, early and late resorptions, and live and dead fetuses were recorded. The reviewer calculated the total pre-implantation loss and the total post-implantation loss from the appropriate values.

k. Fetal Examinations

Each fetus was removed from the uterus and individually identified with a tag, weighed, and observed for gross external alterations. The crown-rump length of late resorptions was measured and the tissue discarded. Every fetus was examined to determine sex and soft tissue alterations. Fetuses were then eviscerated, stained with Alizarin red S, and examined for skeletal changes.

l. Statistical Analysis

Adult and fetal incidence data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution.

Maternal body weights, body weight changes and feed consumption and litter averages for percent male fetuses, percent resorbed conceptuses, fetal body weights, anomaly average data and fetal ossification site data were analyzed using Bartlett's Test for Homogeneity of Variances and the Analysis of Variance when appropriate [i.e., Barlett's Test was not significant ($p \leq 0.05$). If the Analysis of Variance was significant [$p \leq 0.05$], Dunnett's Test was used to identify the statistical significance of the individual groups. If the Analysis of Variance was not appropriate [i.e. Barlett's Test was significant [$p \leq 0.05$], the Kruskal-Wallis Test was used, when less than or equal to 75% ties were present; when more than 75% ties were present, Fisher's Exact Test was used. In cases in which the Kruskal-Wallis Test was statistically significant [$p \leq 0.05$], Dunn's method of Multiple Comparisons was used to identify the statistical significance of the individual groups.

All other Caesarean-sectioning data were evaluated using the procedures previously described for the Kruskal-Wallis Test.

Observations for fetuses of does that aborted, aborted conceptuses and late resorptions were excluded from statistical analysis.

One (1) control group doe, 3 low dosage group does and 2 middle dosage group does had litters consisting of 1 to 4 early absorptions. Such occurrences can abnormally skew the distribution of the data and statistical analysis were made with an without the values for these does and their litters. Because exclusion of the values for the does and their litters did not affect the interpretation of the data, only the tables including these values are provided in the report.

m. Regulatory Compliance:

A Good Laboratory Practice statement and a statement of quality assurance was signed and dated. A statement of No Confidentiality Claims was included.

III. RESULTS

A. Maternal Toxicity

a. Mortality

One 300 mg/kg/day doe was found dead on day 12 of gestation; death was considered to be caused by an intubation accident. The litter of this animal consisted of 6 conceptuses that were presumed to have been cannibalized.

b. Abortions

One (1) doe at 150 mg/kg/day aborted on day 22 of gestation. Four (4) does aborted at 300 mg/kg/day [1 doe on day 19, 1 doe on day 21 and 2 does on day 24 of gestation].

c. Clinical Signs

Ataxia was significantly ($p \leq 0.01$) increased in does at 150 mg/kg/day [18/20] and at 300 mg/kg/day [20/20]when compared to the controls [0/19]. Rales were significantly increased in does at 300 mg/kg/day [4/20] compared to none in the control. Decreased motor activity was noted in 2/8 does at 150 and 300 mg/kg/day, respectively. A small number of animals showed an increased incidence of labored breathing, red and white perinasal substance, dried feces, impaired righting reflex, no feces and a red substance in the cage pan. The clinical observations were was noted on day 9 of gestation and one or more of the signs were generally seen in several does throughout the dosing and post-dosing periods.

d. Body Weight Changes

No differences in relative body weight was seen between the treated vs. control groups on days 0 to 6 (pre-dosage period) of gestation. At 300 mg/kg/day, relative body weight was significantly [$p \leq 0.01$] reduced during the entire dosing period from days 6 to 19 (-0.20 gm

vs. 0.04 gm in the control]. Within this dosage period, a statistically significant ($p \leq 0.05$) relative body weight decrease occurred during days 6 to 7 [-0.07 gm vs. -0.02 gm in the control] (Table 1).

At 300 mg/kg/day, during the post-dosage period (days 19 thru 29 of gestation), a statistically significant ($p \leq 0.05$ to $p \leq 0.01$) increase in absolute body weight occurred in all dosage groups vs. the controls. Relative body weight increased, but was not statistically significant, on days 19 to 29 only at the 300 mg/kg/day dose. Relative body weight was significantly ($p \leq 0.01$) reduced on days 6 to 29 [0.09 gm vs. 0.28 gm in the control] and days 0 to 29 [0.09 gm vs. 0.28 gm in the control] of gestation at 300 mg/kg/day (Table 1).

Table 1. Relative Maternal Body Weight[1]

Gestation Day	Relative Maternal Body Weight (gms) Dose Level (mg/kg/day)			
	0	30	150	300
6 to 7	-0.02	-0.01	-0.04	-0.07*
9 to 12	0.01	0.06	0.03	-0.02
15 to 19	-0.02	0.03	0.03	-0.11
6 to 19	0.04	0.16	0.07	-0.20**
19 to 29	0.24	0.22	0.21	0.32
6 to 29	0.28	0.38	0.29	0.09**
0 to 29	0.45	0.56	0.47	0.26**

[1]Adapted from original report, Vol. 1, p. 51.
*p<0.05
**p<0.01

e. Food Consumption

At 300 mg/kg/day, the absolute [gm/day] feed consumption was significantly [$p \leq 0.05$] reduced [93.2 gm vs. 135.2 gm in the control] during days 6 to 19 of dosing. Absolute feed consumption was also significantly reduced at this dose during days 6-29 and 0-29.

During the post-dosage period (days 19 to 29 of gestation), the relative feed consumption was similar in all 4 dosage groups.

At 300 mg/kg/day, the relative feed consumption (gm/kg/day) was significantly ($p \leq 0.01$) reduced during days 6-29 [29.5 gm vs. 37.0

gm in the control) and during days 0-29 (34.2 gm vs 39.5 gm in the control) while the relative feed consumption was comparable at the mid-dose; does at the low dose exhibited an increase in feed consumption.

f. Macroscopical Examination

No treatment-related macroscopic changes were observed in the dams sacrificed at termination or in the does that aborted.

Necropsy of the doe that died at 300 mg/kg/day on gestation day 12 exhibited a thick, hard and grey esophagus and a white mucoid substance in the trachea. The trachea and esophagus observations was considered to be cause by an intubation accident.

g. Reproduction Data

The pregnancy rate was 94.7%, 80.0%, 90.0% and 90.0% at the 0, 30, 150 and 300 mg/kg/day groups, respectively. Day 29 Caesarean-sectioning observations were based on 18, 16, 18 and 18 pregnant does in the 0, 30, 150 and 300 mg/kg/day dosage groups, respectively, and excluded the 1 doe in the 300 mg/kg/day group that died and the 1 doe at 300 mg/kg/day and 4 does at 150 mg/kg/day that aborted (Table 2).

There were no significant differences among the dosage groups in the litter means for corpora lutea, implantations, litter sizes, resorptions (early and late), percent resorbed conceptuses (early or late), total pre-implantation loss and fetal body weights. The spurious increase in post-implantation loss [15.6%] at 150 mg/kg/day when compared to the controls [7.4%] was caused by an increase in the number of early resorptions. The live male/female ratio and % males/litter [] was 56/57 [49.6%], 52/35 [59.8%], 50/41 [54.9%] and 40/36 [52.6%] at 0, 30, 150 and 300 mg/kg/day, respectively (Table 2).

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Table 2. Cesarean Section Observations[a]

Observations	Incidence of Observations — Dose Level [mg/kg/day]			
	0	30	150	300
# Assigned	19	20	20	20
Females Gravid (%)	18 (94.7)	16 (80.0)	18 (90.0)	18 (90.0)
Maternal Wastage				
# Died (%)	0	0	0	1 (5.6)**
# Aborted (%)	0	0	1 (5.5)	4 (22.2)**
# Non pregnant (%)	0	0	0	0
Corpora Lutea (Mean±SD)	9.6±2.2	8.4±3.2	8.9±2.4	9.2±1.5
Implantations (Mean±SD)	6.8±2.2	5.9±3.1	6.4±3.0	6.3±1.2
Total Live Fetuses (#)	113	87	91	76
Live Fetuses (Mean±SD)	6.3±2.4	5.4±3.5	5.4±3.4	5.8±1.5
Total Dead Fetuses (#)	0	0	0	0
Total Resorptions (#)	9	8	17	6
(#)	7	8	14	4
Early Resorptions (Mean±SD)	0.4±0.6	0.5±0.9	0.8±1.4	0.3±0.6
(#)	2	0	3	2
Late Resorptions (Mean±SD)	0.1±0.3	0.0±0.0	0.2±0.5	0.2±0.4
Pre-implantation Loss[b] (%)	29.2	29.8	28.1	31.5
Post-implantation Loss[b] (%)	7.4	8.5	15.6	7.9
Sex Ratio (♂/♀)	56/57	52/35	50/41	40/36
♂/Litter (%)	49.6	59.8	54.9	52.6
Mean Fetal Weight (gm)	44.6	47.1	44.2	42.5

[a]Adapted from original report, Vol. 1, p. 56.
[b]Calculated by the reviewer, not statistically analyzed.
** $p \leq 0.01$.

B. Developmental Toxicity

Fetal malformations were summarized in Table 3 and fetal variations were summarized in Table 4.

a. External Examinations

No treatment-related or statistically significant external malformations or variations were seen between the control and treated groups (Table 3, 4).

b. Visceral Examinations

No treatment-related or statistically significant soft tissue malformations were seen between the control and treated groups. (Table 3).

Soft tissue variations known to occur spontaneously in rabbits were seen with similar frequency in the control and treated groups (Table 4).

c. Skeletal Examinations

No treatment-related or statistically significant skeletal malformations were seen between the control and the treated groups (Table 3).

An increase was observed in the number of fetuses with common small irregularities in ossification of the skull (the presence of small ossification sites within the sutures or calvaria [nasal, frontal or parietal bones] and/or irregular shaping or fusion of the sutures or bones). The fetus/litter incidence was 23/13, 33/10, 20/10 and 28/13 irregularities, respectively, at the 0, 30, 150 and 300 mg/kg/day dose levels. Statistical significance ($p \leq 0.01$) in the number of fetuses with this lesion occurred at 30 and 300 mg/kg/day (Table 4).

The summary of irregular ossifications of the nasal bones [internasals; irregular suture; midline suture displaced and nasal-frontal irregular suture], presented the following fetus/litter incidences at 0, 30, 150 and 300 mg/kg/day, respectively, 17/11, 20/10, 9/7 and 19/11. The fetal incidence was statistically significant at both 30 ($p \leq 0.05$) and 300 ($p \leq 0.01$) mg/kg/day (Table 4).

When only the nasal bones were examined, statistical significance ($p \leq 0.01$) was seen in both the fetal (3) and litter (3) variations at 300 mg/kg/day vs. 0/0 in the control. The percentage increases were 3.9% for the fetus and 23.1% for the litters vs. 0% at the other dosage levels (Table 4).

No other fetal skeletal alterations were significantly altered relative to the controls.

Table 3. Summary of Fetal Malformations*

Observations	No. of Fetuses / No. of Litters — Dose Level [mg/kg/day]			
	0	30	150	300
No. Examined Externally	113/17	87/13	91/15	76/13
Body:				
Umbilical Hernia	1/1	0/0	0/0	0/0
Meningocele	0/0	0/0	1/1	0/0
Hindlimbs:				
Rotated Medially	0/0	0/0	0/0	1/1
Paws, Flexed	0/0	0/0	0/0	1/1
Tail:				
Short	0/0	0/0	0/0	1/1
No. Examined Viscerally	113/17	87/13	91/15	76/13
Liver:				
Protrudes	1/1	0/0	0/0	0/0
Kidney:				
Displaced Caudally	0/0	1/1	0/0	1/1
No. Examined Skeletally	113/17	87/13	91/15	76/13
Vertebrae:				
Thoracic, Hemivertebra	0/0	2/1	0/0	0/0
Thoracic, Centrum, Unilateral Ossification	0/0	1/2	0/0	0/0
Thoracic, Centrum, Asymmetric	0/0	1/1	0/0	0/0
Thoracic Arch, Small	0/0	2/1	0/0	0/0
Lumbar, Arches, Fused	0/0	0/0	1/1	0/0
Lumbar, Arch, Irregularly Shaped	0/0	0/0	1/1	0/0
Caudal, Misaligned	1/1	0/0	0/0	0/0
Caudal, 14 Present	0/0	0/0	0/0	1/1
Ribs:				
Fused	0/0	1/1	0/0	0/0
Split	0/0	1/1	0/0	0/0
Short	0/0	1/1	0/0	0/0
Unattached	0/0	1/1	0/0	0/0

*Adapted from original report, Vol. 1, p. 59 to 66.

Table 4. Summary of Fetal Variations[a]

Observations	No. of Fetuses / No. of Litters			
	Dose Level [mg/kg/day]			
	0	30	150	300
No. Examined Externally No. With External Variations	113/17 0/0	87/13 0/0	91/15 0/0	76/13 0/0
No. Examined Viscerally	113/17	87/13	91/15	76/13
Lungs, Intermediate Lobe, Agenesis	9/4	5/3	8/5	2/2
Gall Bladder, Agenesis	0/0	1/1	0/0	1/1
No. Examined Skeletally	113/17	87/13	91/15	76/13
Skull, Irregular Ossification (%)	23/13 20.4/76.5	33**/10 37.9/76.9	20/10 22.0/66.7	28**/13 36.8/100
Nasal(s) irregular ossification (summation of Internasals; irregular suture; midline suture displaced; (Nasal-Frontal, Irregular Suture)	17/11	20*/10	9/7	19**/11
(i) Nasals, Internasal (%)	0/0 0	0/0 0	0/0 0	3**/3** 3.9/23.1
Skull, Parietal, contains a small hole	0/0	0/0	0/0	1/1
Hyoid, Ala, Angulated	10/7	4/4	6/5	8/6
Ribs, Thickened Areas of Ossification	1/1	0/0	0/0	0/0
Sternebrae, Fused Sternebrae, Asymmetric	5/3 1/1	0/0 0/0	3/2 1/1	5/4 1/1
Scapulae, Ala, Irregularly Shaped	1/1	0/0	0/0	0/0
Pelvis, Pubes, Not Ossified	0/0	0/0	0/0	1/1

[a] Adapted from original report, Vol 1, p. 59 to 66.
* $p \leq 0.05$
** $p \leq 0.01$

IV. DISCUSSION

Oral administration of Dicamba technical at 0, 30, 150 and 300 mg/kg/day to inseminated rabbits during days 6 to 19 of gestation resulted in significant maternal toxicity manifested by four, 300 mg/kg/day dose groups animals aborting on days: 19 (1 doe), 21 (1 doe) and 24 (2 does) and one (1), 150 mg/kg/day doe aborting on day 22. The abortions were considered to be treatment related as they occurred only at the mid and high-dose groups.

Maternal toxicity was further evidenced at the 150 and 300 mg/kg/day dosage groups by an increased number of animals with decreased motor activity and ataxia. At 300 mg/kg/day, labored breathing, impaired righting reflex, no feces, a red substance in the cage pan and a red or yellow perinasal substance occurred. The clinical observations were considered to be caused by the administration of the test compound for they occurred in the higher dose groups in both the main and the range-finding studies. No maternal toxicity was observed at 30 mg/kg/day.

At 300 mg/kg/day, a relative body weight decrease together with a decrease in the relative maternal feed consumption occurred during the entire dosing period from days 6 to 19 of gestation. Within this dosing period, a relative body weight and relative feed consumption decrease occurred on day 6 to 7. On days 6 to 29 and 0 to 29, a relative body weight decrease occurred together with a decrease in the relative feed consumption.

No treatment-related differences were seen in the caesarian section parameters except for (i) a spurious increase in the post-implantation loss at 150 mg/kg/day [15.6% versus the control (7.4%) caused by an increase (0.8/litter] in early resorptions. This finding was not considered to be of any toxicological significance for a dose relationship was not seen and (ii) the percentage males/litter was higher at all dosage levels vs. the control - 49.6%, 59.8%, 54.9% and 52.6%, respectively, at 0, 30, 150 and 300 mg/kg/day. Since the historical control for the percentage males/litter was 50.5% [25.9 - 100%], this fact suggests that the results fell within the normal range.

The developmental toxicity parameters (external, visceral or skeletal malformations or variations) were similar in the treated vs. the control groups. There was an increase in the number of fetuses with small irregularities of skull ossification sites within the sutures or calvaria [nasal, frontal or parietal bones] and/or irregular shaping or fusion of the sutures or bones occurred) which occurred in the fetus control and treated doses. The statistically significant increases in the fetal incidences of irregular skull ossification and irregular ossification of the nasal bones at the low and the high doses were not considered to be treatment related since (i) the litter incidences of these skeletal variations in these dose groups were similar to that of the

controls, (ii) there was no dose-response, and (iii) the incidences were within the historical control range of the testing laboratory.

The increases observed in irregular ossification of the internasal bone at the high-dose level was attributed to treatment since (i) the alteration was seen only at the high dose; (ii) the increase was statistically significant [$p \leq 0.01$] when compared to the controls; and (iii) both the fetal [3/76, 4%] and litter [3/13, 23%] incidences at the high dose exceed the historical control range [fetal, 0-2.3%; litter 0-14%] [Appendix 1].

V. CONCLUSIONS

Dicamba technical did not induce maternal or developmental toxicity at 30 mg/kg/day. At 150 only maternal toxicity was seen. At 300 mg/kg/day, maternal and developmental toxicity occurred. Maternal toxicity was characterized by clinical signs, abortions and decreases in body weight gain and food consumption. Developmental toxicity was manifested by irregular ossification in the nasal bones [internasals].

Based on the results of this study the following NOELs and LOELs are established:

Maternal Toxicity

NOEL: 30 mg/kg/day
LOEL: 150 mg/kg/day

Developmental Toxicity

NOEL: 150 mg/kg/day
LOEL: 300 mg/kg/day

VI. CORE CLASSIFICATION:

Minimum; this study satisfies the data requirements 83-3(b) for a Developmental Toxicity Study in rabbits and is acceptable for regulatory purposes.

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Appendix 1

Historical Control Values for Selected Skull Ossification Irregularities*

Period: 1987-1969
No. of Studies: 32
No. of Litters examined: 428
No.of Fetuses examined: 3088

Alterations	L/F	N	%	Range N	Study %	No. of Studies with Alterations
Skull:						
Summarization of all irregular ossification	L F	388 1061	90.65 34.36	2-18 13-63	57.1-100 14.0-73.7	32
Nasals, irregular	L	17	3.97	0-2	0-14.3	13
(i) Internasals	F	18	0.58	0-2	0-2.3	

*Adapted from original report, Vol. 2, p. 338, 339.

END